PADEMAN

TRANSMITTAL OF APPEAL BRIEF (Small Entity)

Application No. Filing Date Examiner Customer No. Group Art Unit Confirmation No. 09/981,900 10/18/01 Russell Kallis 21036 9143 1638

Invention:

TRANSGENIC PLANTS CONTAINING LIGNINASE AND CELLULASE WHICH DEGRADE LIGNIN AND CELLULOSE TO FERMENTABLE SUGARS

COMMISSIONER FOR PATENTS:

Transmitted herewith in triplicate is the Appeal Brief in this application, with respect to the Notice of Appeal filed on: September 7, 2005

Applicant claims small entity status. See 37 CFR 1.27

The fee for filing this Appeal Brief is:

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Dated: 9/29/2005

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicants : Masomeh B. Sticklen, Bruce E. Dale and

Shahina B. Maqbool

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Examiner : Kallis, Russell

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^{250.00 OP}BRIEF UNDER 37 C.F.R. § **41**.37

Sir:

This is an appeal from a final rejection in the above entitled application. The claims on appeal are set forth as Claims Appendix. An oral hearing will be requested. Enclosed is the fee due upon filing of the Brief.

(1) Real Party in Interest

The real party in interest is the Board of Trustees operating Michigan State University, East Lansing, Michigan, a constitutional corporation of the State of Michigan, which is the assignee of the above entitled application.

(2) Related Appeals and Interferences

There are no pending related appeals and interferences.

(3) Status of Claims

Claims 2-6, 16, 18-46, 48-52, 62, 66-70, 80, 83-99, 101 and 104 have been cancelled. Claims 1, 7-15, 17, 47, 53-61, 63-65, 71-79, 81-82, 100, 102 and 103 are pending in the application. All claims were rejected. No claims have been allowed. Claims 1, 7-15, 17, 47, 53-61, 63-65, 71-79, 81-82, 100, 102 and 103 are on appeal.

(4) Status of Amendments

No amendments subsequent to final rejection have been filed.

(5) Summary of Claimed Subject Matter

The claimed subject matter in Claim 1 is herbaceous transgenic plant which degrades lignocellulose when the transgenic plant is ground to produce a plant material comprising: (a) at least one DNA encoding a cellulase wherein this one DNA is comprised of DNA sequences selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to a plastid or apoplast of the transgenic plant; and (b) at least one DNA encoding a ligninase comprising a lignin peroxidase gene, wherein this one DNA is comprised of DNA in sequences selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 13 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to the plastid or

apoplast of the transgenic plant, wherein the transgenic plant degrades the lignocellulose when ground to produce the plant material.

Support for this is found in original Claim 1 and line 25 through page 9, line 3 of at page 8, the Support for DNA encoding a cellulase is specification. disclosed as the sequences SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10 described at page 23, line 27 through page 24, line 16 of the specification. SEQ ID NO: 4 (GenBank Accession No. U33212) provides the nucleotide sequence for the gene encoding EI beta-1,4-endoglucanase precursor (e1) from Acidothermus cellulolyticus. NO:6 provides the nucleotide sequence of the glucosidase gene from Actinomyces naeslundi (GenBank Accession No. AY029505). SEQ ΙD NO:8 provides the nucleotide sequence of the dextranase gene Streptococcus salivarius (GenBank Accession No. D29644), encodes a glucanhydrolase which SEQ ID NO: 10 is nucleotide sequence for the encoding gene cellulase cellobiohydrolase (cbh1) from Trichoderma reesi. for operably linking these to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the

cellulase to a plastid or apoplast of the transgenic plant is described in Example 2, starting on page 43. The plasmid DNA constructs are summarized in Table 2. The signal peptide that directs the protein to which it is attached to the plant organelle, the chloroplasts, are the nucleotide codons that encode the rice rubisco synthase gene (rbcS) small subunit signal peptide (rbcSSP) (page 22, line 30 to page 23, line 2). The nucleotide sequence of the rbcS is set forth in SEQ ID NO:1 (GenBank Accession No. X07515). The signal peptide of construct 5 in Table 2 is encoded by DNA encoding the soybean vegetative storage protein betaleader (VSP) signal peptide which target the cellulase to the apoplasts (page 44, line 31 to page 45, line 4).

Support for DNA encoding a ligninase comprising a lignin peroxidase gene, wherein this one DNA is comprised of DNA in sequences selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 13 described at page 25, lines 23-34. The nucleotide sequence of the *ckg4* ligninase gene is set forth in SEQ ID NO:11 (GenBank Accession No. M18743). The nucleotide sequence of the *ckg5* ligninase gene is set forth in SEQ ID NO:13 (GenBank Accession No. M18794). As described in Example 3, starting at page 48, these genes are

also operably linked to a nucleotide sequence encoding a signal peptide. Transformation of maize is described in Example 5, starting at page 53 of the specification.

The claimed subject matter in Claim 47 is a method for producing a herbaceous transgenic plant which degrades lignocellulose when the transgenic plant is ground to produce a plant material comprising: (a) providing a first transgenic plant which includes a DNA encoding a cellulase, wherein this one DNA is comprised of DNA in sequences wherein the DNA encoding the cellulase is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to a plastid or apoplast of the transgenic plant and a second transgenic plant which includes a DNA encoding a ligninase comprising a lignin peroxidase gene wherein this one DNA is comprised of DNA in sequences' selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 13 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to the plastid or

apoplast of the transgenic plant; and (b) mating by sexual fertilization the first and the second transgenic plants to produce a third transgenic plant which includes the first DNA encoding the cellulase and the second DNA encoding the the transgenic plant the ligninase, wherein degrades lignocellulose when ground to produce the plant material. Support for this is described above for Claim 1 and also Methods of cross-breeding found in original Claim 47. transgenic plants (step b), one having a cellulase and the other a ligninase stably integrated into the genome are in Example 6, starting at page 55 of the described specification.

The claimed subject matter in Claim 65 is a method for converting lignocellulose in a herbaceous plant material to fermentable sugars comprising: (a) providing a herbaceous transgenic plant which includes at least one DNA encoding a cellulase, wherein this one DNA is comprised of DNA in sequences selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the

cellulase to a plastid or apoplastid of the transgenic plant and a at least one DNA encoding a ligninase comprising a lignin peroxidase gene wherein this one DNA is comprised of DNA in sequences selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 13 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to the plastid or the transgenic plant; (b) growing apoplastid of transgenic plant for a time sufficient for the transgenic plant to accumulate a sufficient amount of the cellulase and the ligninase in the plastid or apoplastid of the transgenic harvesting the transgenic plant which (c) accumulated the cellulase and ligninase in the plastid or the transgenic plant; (d) grinding apoplastid of transgenic plant for a time sufficient to produce the plant material wherein the cellulase and ligninase produced by the transgenic plant are released from the plastid or apoplastid of the transgenic plant; (e) incubating the plant material for a time sufficient for the cellulase and ligninase in the plant material to produce the fermentable sugars from the lignocellulose in the plant material; and (f) extracting the fermentable sugars produced from the lignocellulose by the

cellulase and the ligninase from the plant material. Support for this is as described for Claim 1 above and in original Claim 65.

(6) Grounds of Rejection to Be Reviewed on Appeal

(a) Claims 1, 7-15, 17, 47, 53-61, 63-65, 71-79, 81-82, 100, 102 and 103 were rejected under 35 U.S.C. \$103(a) as being unpatentable over Himmel et al. (U.S. Patent No. 6,013,860 issued January 11, 2000), in view of Crawford et al. (U.S. Patent No. 5,200,338 issued April 6, 1993), and in further view of de Boer et al. (Gene, 1987, vol. 60, pp. 93-102), and Applicants admissions of the prior art.

(7) Argument

(a) Are the claimed transgenic plants having SEQ ID defined DNA encoding a cellulase and DNA encoding a ligninase obvious when the prior art does not show or suggest transgenic plants having DNA encoding a ligninase, nor show or suggest transgenic plants having a combination of DNA encoding a cellulase and DNA encoding a ligninase?

The Examiner rejected Claims 1, 7-15, 17, 47, 53-61, 63-65, 71-79, 81-82, 100, 102 and 103 under 35 U.S.C. \$103(a) as being unpatentable over Himmel et al. (U.S. Patent No. 6,013,860), in view of Crawford et al. (U.S. Patent No. 5,200,338), and in further view of de Boer et al. (Gene, 1987, vol. 60, pp. 93-102), and Applicants' admissions of the prior art.

Himmel et al. discloses engineered plant cells having a DNA for a polysaccharide hydrolyzing enzymes (cellulases, cellobiohydrohydrolases, xylanases, hemicellulases) integrated into the plant cell plastid genome. Himmel et al. also teach integrating DNA for the cellulase into the host plant nuclear genome and targeting the expressed enzyme to a cellular organelle such as a

plastid. Himmel et al. teaches targeting the expressed enzyme to the plastid by means of a plastid transit peptide and the use of marker genes which overcome a natural inhibition by, attenuate or inactivate a selective substance such as antibiotics or herbicides for the selection of desired plant cells. Himmel et al., however, does not show suggest a transgenic plant having DNA encoding cellulase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to an apoplast of the transgenic plant. et al. does not show or suggest a transgenic plant having DNA encoding a ligninase. Himmel et al. does not show or suggest a transgenic plant having DNA encoding a ligninase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to an apoplast of the transgenic plant.

Enzymatic methods of converting lignocellulose in a plant material to fermentable sugars, GenBank Accession Number X07515 having a plastid signal peptide sequence of the *rbc*S gene as set forth in SEQ ID NO: 2, and the *bar* gene were all known in the art as stated in the Applicant's specification. Crawford *et al.* teach removing lignin from

lignocellulose for lignocellulose biodegradation. Lignin is a polymer of an aromatic alcohol which represents a class of substances that bind together cellulose fibers which make up a substantial portion of the woody portions of plant tissue (Crawford et al.: col. 1, lines 28-31). Additionally, Crawford et al. teach a lignin peroxidase enzyme obtained from a bacterial source which is capable of degrading the lignin of lignocellulose. De Boer et al. teach lignin peroxidase (LIP) genes CLG4 (H2, ckg4), as set forth in SEQ ID NO: 11, and CLG5 (H10, ckg5), as set forth in SEQ ID NO: 13. De Boer et al. and Crawford et al., taken alone or in combination, do not show or suggest a transgenic plant having DNA encoding a ligninase, such as ckg4 or ckg5, either with or without DNA encoding a cellulase. De Boer et al. and Crawford et al., taken alone or in combination, do not show or suggest a transgenic plant having DNA encoding a ligninase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to a plastid or apoplast of the transgenic plant.

To establish a prima facie case of obviousness, there must be some suggestion or motivation, either in the

references knowledge themselves or in the generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. According to M.P.E.P. §2143.01 the prior art must suggest "There are three desirability of the claimed invention. possible sources for a motivation to combine references: the nature of the problem to be solved, the teachings of the prior art, and the knowledge of persons of ordinary skill in the art." In re Rouffet, 149 F.3d 1350, 1357, 47 USPQ2d 1453, 1457-58 (Fed. Cir. 1998) (The combination of the references taught every element of the claimed invention, however without a motivation to combine, a rejection based on a prima facie case of obvious was held improper.). level of skill in the art cannot be relied upon to provide the suggestion to combine references. Al-Site Corp. v. VSI Int'l Inc., 174 F.3d 1308, 50 USPQ2d 1161 (Fed. Cir. 1999).

The fact that the claimed invention is within the capabilities of one of ordinary skill in the art is not sufficient by itself to establish prima facie obviousness. It is not sufficient to establish a prima facie case of obviousness that the modifications to meet the claimed invention would have been "well within the ordinary skill of

the art" at the time the invention was made, even if the references teach that all aspects of the claimed invention were individually well known in the art. This alone is not sufficient to establish a prima facie case of obviousness without some objective reason to combine the teachings of the references. Ex parte Levengood, 28 USPQ2d 1300 (Bd. Pat. App. & Inter. 1993). See also In re Kotzab, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1318 (Fed. Cir. 2000) (Court reversed obviousness rejection involving technologically simple concept because there was no finding as to principle or specific understanding within the knowledge of skilled artisan that would have motivated the skilled artisan to make the claimed invention); Al-Site Corp. v. VSI Int'l Inc., 174 F.3d 1308, 50 USPQ2d 1161 (Fed. Cir. 1999) (The level of skill in the art cannot be relied upon to provide the suggestion to combine references.). That one can reconstruct and/or explain the theoretical mechanism of an invention by means of logic and sound scientific reasoning does not afford the basis for an obviousness conclusion unless that logic and reasoning also supplies sufficient impetus to have led one of ordinary skill in the art to combine the teachings of the references to make the

claimed invention. Ex parte Levengood, 28 USPQ2d at 1302.

In the present rejection, it is stated that one of skill in the art would have been motivated by the knowledge common in the art that ligninase gene products are valuable materials for breaking down lignocellulose fermentables, such as taught by Crawford et al. at column 1, lines 38-54. Even if a person of ordinary skill in the art aware that ligninase gene products, are valuable materials for breaking down lignocellulose into fermentables and it was within the capabilities of one of ordinary skill in the art to make the invention from the known individual obviousness elements of the invention, has not established. The cited references still do not provide sufficient impetus to lead one of ordinary skill in the art to provide a transgenic plant having a DNA encoding a ligninase comprising a lignin peroxidase gene such that lignocellulose is degraded when the transgenic plant ground.

The teaching or suggestion which motivates the person of skill in the art to make the claimed combination must be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed.

Cir. 1991). Himmel et al. does not teach or suggest degrading lignin, and even if Crawford et al. motivated a person of skill in the art to degrade lignin to allow for the efficient use of cellulosic material, Crawford et al. would suggest to the person of skill in the art to utilize a simple and effective enzyme preparation or lysate derived lines from microbial source (col. 6, Alternatively, Crawford et al. teach using other prokaryotic hosts by means of DNA technology (col. 7, lines 49 through col. 8, line 55) as the best alternative to fungal lignin degradation.

According to Crawford et al.,

"[t]he bacterial source and the enzyme itself offer over the previously available advantages The bacterial enzyme is enzymes. expressed as primary metabolite, is inducible by substances other lignin, and it can attack certain lignin chemical substructure bonds that fungal lignin peroxidases cannot. The use of bacteria versus fungal systems offers other advantages, particularly in using actinomycetes. There is a strong background expertise in large-scale cultivation of actinomyctes on a variety of substrates for commercial antibiotic production. Further, lignin degradation is subject to stringent physiological ligninolytic activity than white-root rot fungi. As disclosed earlier, improvement strain using the recombinant techniques can be readily applied to actinomycetes. In contrast, strain improvement by genetic manipulation less attainable with fungi. Bacteria are more easily manipulated and it would be simple to genetically engineer bacterial strains

overproduce the enzyme, while this is a difficult task in fungi. In fungi, the gene must be transferred to a bacterium for genetic manipulation and expression. Even then, the genes are not always expressed in bacteria. Therefore, the use of the bacterial enzyme has the potential to overcome the problems of insufficient yield in ligninolytic systems."

(Crawford et al. col. 13, 39-63). Therefore, Crawford et al. teach that bacteria are the preferred source for the ligninase enzyme. Genetic manipulation of bacteria is clearly preferred by Crawford et al. to that of other organisms due to the ease of manipulation. A person of ordinary skill in the art, reading Crawford et al. would not be motivated to pursue a transgenic plant having a DNA encoding a ligninase.

According to <u>Crawford</u> et al., one advantage of the bacterial enzyme is that the enzyme is extracellular. When the bacteria is grown in culture, the enzyme occurs in the culture medium (col. 4, lines 19-22). Additionally, the lignin peroxidase enzyme is associated with primary growth of the bacteria. <u>Crawford</u> et al. teaches that it is presumed to be the result of primary metabolic activity and not dependent upon other factors such as stress to induce production. Thus, the ligninase is easy to produce and purify. A person of ordinary skill in the art, after

reading of these advantages of a bacterial enzyme, would not be led to pursue a transgenic plant having a DNA encoding a Crawford et al. teach that the bacterial lignin peroxidase can be prepared simply as a crude extract by growth of suitable bacteria in appropriate medium described in Column 4, lines 23-43. Crawford et al. also ligninase activity teach methods of enhancing the addition of larch wood xylan to the bacterial culture, and also that the enzyme can be further purified by conventional purification techniques (col. 4, line 44 through col. 5, Therefore, a person of ordinary skill in the art line 4). would be led by Crawford et al. to use bacterial sources of ligninase enzyme, such as actinomycetes in the and particular Streptomyces (col. 5, lines 17-33).

Crawford et al. suggests that recombinant bacterial strains can be used to improve enzyme production because of the ease of modification of the bacterial genomes (col. 8, lines 38-41). A person of ordinary skill in the art would not be motivated by the teaching of Crawford et al. to attempt insertion of the ligninase gene directly into herbaceous plants, since a person skilled in the art after Crawford would only be motivated reading et al.

genetically modify bacteria to improve enzyme production by recombinant techniques, due to the ease of genetically modifying bacteria. The references would not sufficient impetus to incorporate the ligninase genes into herbaceous plants which are more difficult to genetically modify, when Crawford et al. teaches the advantages of recombinant bacterial strains enzyme and preparations. There is no suggestion of any advantage to attempt such a difficult approach when enzymes and bacterial strains can be so easily manipulated as fully described in Crawford et al. (col. 7, line 15 through col. 8, line 55).

Therefore, <u>Crawford</u> et al. teaches away from incorporating the ligninase genes into herbaceous plants, by teaching simple and effective bacterial solutions to the problem of degrading lignin. Nothing in <u>Crawford</u> et al. taken alone or in combination with the other references gives a person of skill in the art impetus to even try the claimed invention. <u>Himmel</u> et al., <u>Crawford</u> et al., and <u>de</u> <u>Boer</u> et al., either taken alone or in combination, do not show or suggest the limitations of the claimed invention. In light of the these arguments Claims 1, 7-15, 17, 47, 53-61, 63-65, 71-79, 81-82, 100, 102 and 103 are patentable

over <u>Himmel</u> et al., <u>Crawford</u> et al., and <u>de Boer</u> et al., either taken alone or in combination.

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requested.

(b) Conclusion

As shown above, the claimed invention is not obvious over <u>Himmel</u> et al. in view of <u>Crawford</u> et al., and further in view of <u>de Boer</u> et al. Therefore, Claims 1, 7-15, 17, 47, 53-61, 63-65, 71-79, 81-82, 100, 102 and 103 are each patentable. Reversal of the Final Rejection is

Respectfully,

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CLAIMS APPENDIX

- 1. A herbaceous transgenic plant which degrades lignocellulose when the transgenic plant is ground to produce a plant material comprising:
- (a) at least one DNA encoding a cellulase wherein this one DNA is comprised of DNA in sequences selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to a plastid or apoplast of the transgenic plant; and
- (b) at least one DNA encoding а ligninase comprising a lignin peroxidase gene, wherein this one DNA is comprised of DNA in sequences selected from the consisting of SEQ ID NO: 11 and SEQ ID NO: 13 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to the plastid or apoplast of the transgenic plant,

wherein the transgenic plant degrades the lignocellulose when ground to produce the plant material.

- 7. The transgenic plant of Claim 1 wherein the DNA encoding the cellulase and the DNA encoding the ligninase are each operably linked to a leaf-specific promoter.
- 8. The transgenic plant of Claim 7 wherein the leaf-specific promoter is a promoter for rbcS.
- 9. The transgenic plant of Claim 1 wherein the nucleotide sequence encoding the signal peptide encodes a signal peptide of rbcS.
- 10. The transgenic plant of Claim 8 or 9 wherein the rbcS comprises the nucleotide sequence set forth in SEQ ID NO:1.
- 11. The transgenic plant of Claim 1 selected from the group consisting of maize, wheat, barley, rye, hops, hemp, rice, potato, soybean, sorghum, sugarcane, clover, tobacco, alfalfa, and arabidopsis.

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12. The transgenic plant of Claim 1 wherein the DNA

encoding the cellulase and the DNA encoding the ligninase are

stably integrated into nuclear or plastid DNA of the

transgenic plant.

13. The transgenic plant of Claim 1 wherein transgenic

plant further includes a DNA encoding a selectable marker

operably linked to a constitutive promoter.

14. The transgenic plant of Claim 13 wherein the DNA

encoding the selectable marker provides the transgenic plant

with resistance to an antibiotic, an herbicide, or to

environmental stress.

15. The transgenic plant of Claim 14 wherein the DNA

encoding resistance to the herbicide is a DNA encoding

phosphinothricin acetyl transferase which confers resistance

to the herbicide phosphinothricin.

17. The transgenic plant of Claim 1 wherein the plastid

of the transgenic plant is a chloroplast.

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- 47. A method for producing a herbaceous transgenic plant which degrades lignocellulose when the transgenic plant is ground to produce a plant material comprising:
- providing a first transgenic plant (a) includes a DNA encoding a cellulase, wherein this one DNA is comprised of DNA in sequences wherein the DNA encoding the cellulase is selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to a plastid or apoplast of the transgenic plant and a second transgenic plant which includes a DNA encoding a ligninase comprising a lignin peroxidase gene wherein this one DNA is comprised of DNA in sequences selected from the consisting of SEQ ID NO: 11 and SEQ ID NO: 13 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to the plastid or apoplast of the transgenic plant; and
- (b) mating by sexual fertilization the first and the second transgenic plants to produce a third transgenic plant which includes the first DNA encoding the cellulase and the second DNA encoding the ligninase,

wherein the transgenic plant degrades the lignocellulose when ground to produce the plant material.

- 53. The method of Claim 47 wherein the DNA encoding the cellulase and the DNA encoding the ligninase are each operably linked to a leaf-specific promoter such as a promoter for rbcS.
- 54. The method of Claim 53 wherein the leaf-specific promoter is a promoter for *rbc*S.
- 55. The method of Claim 47 wherein the nucleotide sequence encoding the signal peptide encodes a signal peptide of rbcS.
- 56. The method of Claim 54 or 55 wherein the *rbc*S comprises the nucleotide sequence set forth in SEQ ID NO:1.
- 57. The method of Claim 47 selected from the group consisting of maize, wheat, barley, rye, hops, hemp, rice, potato, soybean, sorghum, sugarcane, clover, tobacco, alfalfa, and arabidopsis.

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58. The method of Claim 47 wherein the DNA encoding the

cellulase and the DNA encoding the ligninase are stably

integrated into nuclear or plastid DNA of the transgenic

plant.

59. The method of Claim 47 wherein the first, second,

or both transgenic plants further includes a DNA encoding a

selectable marker operably linked to a constitutive promoter.

60. The method of Claim 59 wherein the DNA encoding the

selectable marker provides the transgenic plant with

resistance to an antibiotic, an herbicide, or to

environmental stress.

61. The method of Claim 60 wherein the DNA encoding

resistance to the herbicide is a DNA encoding

phosphinothricin acetyl transferase which confers resistance

to the herbicide phosphinothricin.

63. The method of Claim 47 wherein the plastid of the

transgenic plant is a chloroplast.

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- The method of Claim 47 wherein progeny of the third transgenic plant are mated by sexual fertilization to a transgenic plant selected from the group consisting of the first, second, and third transgenic plants to produce a transgenic plant comprising multiples of genes encoding cellulases and ligninases.
- 65. A method for converting lignocellulose in a herbaceous plant material to fermentable sugars comprising:
- (a) providing a herbaceous transgenic plant which includes at least one DNA encoding a cellulase, wherein this one DNA is comprised of DNA in sequences selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 10 which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to a plastid or apoplastid of the transgenic plant and a at least one DNA encoding a ligninase comprising a lignin peroxidase gene wherein this one DNA is comprised of DNA in sequences selected from the group consisting of SEQ ID NO: 11 and SEQ ID NO: 13 which is

operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to the plastid or apoplastid of the transgenic plant;

- (b) growing the transgenic plant for a time sufficient for the transgenic plant to accumulate a sufficient amount of the cellulase and the ligninase in the plastid or apoplastid of the transgenic plant;
- (c) harvesting the transgenic plant which has accumulated the cellulase and ligninase in the plastid or apoplastid of the transgenic plant;
- (d) grinding the transgenic plant for a time sufficient to produce the plant material wherein the cellulase and ligninase produced by the transgenic plant are released from the plastid or apoplastid of the transgenic plant;
- (e) incubating the plant material for a time sufficient for the cellulase and ligninase in the plant material to produce the fermentable sugars from the lignocellulose in the plant material; and
- (f) extracting the fermentable sugars produced from the lignocellulose by the cellulase and the ligninase from the plant material.

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71. The method of Claim 65 wherein DNA encoding the cellulase and the DNA encoding the ligninase are each operably linked to a leaf-specific promoter.

- 72. The transgenic plant of Claim 71 wherein the leaf-specific promoter is a promoter for *rbc*S.
- 73. The method of Claim 65 wherein the nucleotide sequence encoding the signal peptide encodes a signal peptide of rbcS.
- 74. The method of Claim 72 or 73 wherein the *rbc*S comprises the nucleotide sequence set forth in SEQ ID NO:1.
- 75. The method of Claim 65 selected from the group consisting of maize, wheat, barley, rye, hops, hemp, rice, potato, soybean, sorghum, sugarcane, clover, tobacco, alfalfa, and arabidopsis.

- 76. The method of Claim 65 wherein the first and second DNAs are stably integrated into nuclear or plastid DNA of the transgenic plant.
- 77. The method of Claim 65 wherein transgenic plant further includes a DNA encoding a selectable marker operably linked to a constitutive promoter.
- 78. The method of Claim 77 wherein the DNA encoding the selectable marker provides the transgenic plant with resistance to an antibiotic, an herbicide, or to environmental stress.
- 79. The method of Claim 78 wherein the DNA encoding resistance to the herbicide is a DNA encoding phosphinothricin acetyl transferase which confers resistance to the herbicide phosphinothricin.
- 81. The method of Claim 65 wherein the plastid of the transgenic plant is a chloroplast.

- 82. The method of Claim 65 wherein the plant material further includes a plant material made from a non-transgenic plant.
- 100. The transgenic plant of Claim 1 wherein the lignocellulose is degraded to fermentable sugars.
- 102. The method of Claim 47 wherein the lignocellulose is degraded to fermentable sugars.
- 103. The method of Claim 65 wherein the fermentable sugars are fermented to ethanol.